

Application No. 10/561,430  
Amdt. Dated: March-21-2011  
Reply to Office Action: Dec-21-2010

2

Amendments to the Claims:

1. (Currently amended) A method of forming a canola protein isolate having a protein content of at least about 90 wt% (N x 6.25) from intact canola oil seeds, which comprises:

heat treating the intact canola oil seeds at a temperature of approximately 90°C for about to 5 to about 10 minutes to deactivate enzymes therein,

dehulling the heat-treated canola oil seeds,  
removing canola oil from the heat treated and dehulled oil seeds to provide a canola oil seed meal, and

processing the canola oil seed meal to recover therefrom the canola protein isolate by the steps:

(i) extracting the canola oil seed meal with an aqueous salt solution to cause solubilization of canola protein in said the canola protein seed meal to form an aqueous canola protein solution having a pH of about 5 to about 8,

(ii) separating the aqueous protein solution from residual canola oil seed meal,

(iii) increasing the protein concentration of said aqueous protein solution while maintaining the ionic strength substantially constant by using a selective membrane technique to provide a concentrated protein solution, preferably by ultrafiltration to produce a concentrated protein solution having a protein concentration of at least about 200 g/L,

(iv) diluting the concentrated canola protein isolate into chilled water having a temperature of below about 15°C to cause the formation of discrete protein particles in the aqueous phase in the form of micelles,

(v) settling the protein micelles to form an amorphous, sticky, gelatinous, gluten-like protein micellar mass, and

Application No. 10/561,430  
Amdt. Dated: March-21-2011  
Reply to Office Action: Dec-21-2010

## 3

(vi) recovering the protein micellar mass from supernatant, the protein micellar mass having a protein content of at least about 90 wt%, as determined by Kjeldahl nitrogen x6.25 on a dry weight basis.

2. (Previously presented) The method of claim 1 wherein said heat treated and dehulled oil seeds are flaked prior to said oil removal step.
3. (Previously presented) The method of claim 1, wherein said canola oil seed meal is provided by:
  - heat treating the intact canola oil seeds to inactivate enzymes therein,
  - cooling the heat treated canola oil seeds,
  - cracking the hulls of the heat treated canola oil seeds,
  - removing cracked hulls from canola seeds, and
  - removing canola oil from the canola meats by solvent extraction to leave the meal.
4. (Previously presented) The method of claim 3 wherein an overs fraction and an unders fraction are separated from the cracked hulls, the overs fraction is recycled to the cracking and separation steps, the unders fraction is subjected to air aspiration for further removal of hulls and the recycled overs fraction and/or air aspirated unders fraction are flaked prior to said solvent extraction step.
5. to 7. (Cancelled)
8. (Previously presented) The method of claim 1 wherein the canola oil seed meal is processed to recover therefrom a canola protein isolate having a protein content of at least about 100 wt% (N x 6.25).
9. (Previously presented) The method of claim 1 wherein said inactivation is effected by heating using steam.
10. (Previously presented) The method of claim 1 wherein said inactivation is effected by heating using radio frequency radiation.

Application No. 10/561,430  
Amdt. Dated: March-21-2011  
Reply to Office Action: Dec-21-2010

11. (Cancelled)

12. (Currently amended) The method of ~~claim 11~~ claim 1 wherein said steps (i) to (vi) are effected on a batch basis and said extracting of said oil seed meal is effected using an aqueous salt solution having an ionic strength of at least about 0.10, ~~preferably about 0.15 to about 0.6~~, and a pH of about 5 to about 6.8, ~~preferably about 5.3 to about 6.2~~, and said aqueous protein solution has a protein content of about 5 to about 40 g/L, ~~preferably about 10 to about 30 g/L~~.

13. (Previously presented) The method of claim 12 wherein said extracting of said oil seed meal is effected with agitation of said aqueous salt solution for about 10 to about 60 minutes, while the concentration of oil seed meal in said aqueous salt solution during said extracting step is about 5 to about 15% w/w.

14. (Currently amended) The method of ~~claim 11~~ claim 1 wherein said steps (i) to (vi) are effected on a continuous basis and said extraction step is effected by:

(i) continuously mixing ~~an oil~~ said oil seed meal with an aqueous salt solution having an ionic strength of at least about 0.10, ~~preferably about 0.15 to about 0.6~~, and a pH of about 5 to about 6.8, ~~preferably about 5.3 to about 6.2~~, at a temperature of about 5° to about 65°C, ~~preferably at least about 35°G~~, and

(ii) continuously conveying said mixture through a pipe while extracting protein from the oil seed meal to form an aqueous protein solution having a protein content of about 5 to about 40 g/L, ~~preferably about 10 to about 30 g/L~~, in a period of time up to about 10 minutes.

15. (Previously presented) The method of claim 14 wherein the concentration of oil seed meal in said aqueous salt solution in said mixing step is about 5 to about 15% w/v.

16. (Currently amended) The method of ~~claim 11~~ claim 1 wherein said extracting of said oil seed meal is effected using an aqueous salt solution having an ionic strength of at least about 0.10, ~~preferably about 0.15 to about 0.6~~, and a pH of about 3 to

Application No. 10/561,430  
Amdt. Dated: March-21-2011  
Reply to Office Action: Dec-21-2010

## 5

about 5 or about 6.8 to about 9.9 and, following said separation of the aqueous protein solution from residual oil seed meal, the pH of the aqueous protein solution is adjusted to a pH of about 5 to about 6.8, preferably about 5.3 to about 6.2.

17. (Currently amended) The method of claim 11 claim 1 wherein following said separating of the aqueous protein solution from the residual canola seed meal, the aqueous protein solution is subjected to a pigment removal step, ~~wherein said pigment removal step preferably is effected by:~~

- (a) ~~diafiltration of the aqueous protein solution;~~
- (b) ~~by mixing a pigment adsorbing agent with the aqueous protein solution and subsequently removing the pigment adsorbing agent from the aqueous protein solution.~~

18. (Currently amended) The method of claim 11 claim 1 wherein said oil seed meal is extracted with water and subsequent thereto salt is added to the resulting aqueous protein solution to provide an aqueous protein solution having an ionic strength of at least about 0.10.

19. (Currently amended) The method of claim 11 claim 1 wherein said concentrated protein solution is warmed to a temperature of at least about 20°C, preferably about 25°C to about 40°C, to decrease the viscosity of the concentrated protein solution but not beyond a temperature above which the temperature of the concentrated protein solution does not permit micelle formation.

20. (Currently amended) The method of claim 11 claim 1 wherein said concentrated protein solution is subjected to diafiltration using an aqueous salt solution of the same molarity and pH as the extracting solution, preferably using from about 2 to about 20 volumes, preferably about 5 to about 10 volumes of diafiltration solution, preferably until no further quantities of phenolics and visible colour are present in the permeate.

Application No. 10/561,430  
Arndt. Dated: March-21-2011  
Reply to Office Action: Dec-21-2010

## 6

21. (Currently amended) The method of claim 20 wherein an anti-oxidant, preferably sodium sulfite or ascorbic acid, is present in the diafiltration medium, preferably in an amount of about 0.01 to about 1 wt%, during at least part of the diafiltration step.
22. (Currently amended) The method of ~~claim 11~~ claim 1 wherein the concentrated protein solution, optionally diafiltered, is subjected to a pigment removal step, preferably using powdered activated carbon in an amount of about 0.025 to about 5% w/v, preferably about 0.05 to about 2% w/v, or using polyvinylpyrrolidone in an amount of about 0.5 to about 5% w/v, preferably about 2 to about 3% w/v.
23. (Currently amended) The method of ~~claim 11~~ claim 1 wherein the concentrated protein solution, optionally diafiltered, is subjected to a pasteurization step, preferably by heating the concentrated and optionally diafiltered protein solution at a temperature of about 55° to about 70°C, preferably about 60° to about 65°C, for about 10 to about 15 minutes, and wherein, following said pasteurization step, the pasteurized solution preferably is cooled to a temperature of about 25° to about 40°C.
24. (Currently amended) The method of ~~claim 11~~ claim 1 wherein said steps (i) and (vi) are effected in a batch mode of operation and said concentrated protein solution is diluted by about 15 fold or less, preferably 10 folds or less, by adding the concentrated protein solution into a body of chilled water having the volume required to achieve the desired degree of dilution and preferably having a temperature of less than about 10°C.
25. (Currently amended) The method of ~~claim 11~~ claim 1 wherein said steps (i) to (vi) are carried out in a continuous mode and said concentrated protein solution is continuously mixed with said chilled water to provide a dilution of the concentrated protein solution by about 15 fold or less, preferably about 10 fold or less, and preferably having a temperature of less than about 10°C.

Application No. 10/561,430  
Amdt. Dated: March-21-2011  
Reply to Office Action: Dec-21-2010

26. (Currently amended) The method of claim 14 claim 1 wherein the recovered protein micellar mass is dried to a proteinaceous powder.

27. (Currently amended) A method of forming two different canola protein isolates having a protein content of at least about 90 wt% ( $N \times 6.25$ ) d.b. from intact canola seeds, which comprises:

- (a) heating treating the intact canola oil seeds to deactivate enzymes therein,
- (b) dehulling the heat treated canola oil seeds,
- (c) removing canola oil from the heat treated and dehulled oil seeds to provide a canola oil seed meal,
- (d) extracting the canola oil seed meal with an aqueous salt solution to cause solubilization of canola protein in said the canola protein seed meal to form an aqueous canola protein solution having a pH of about 5 to about 6.8,
- (e) separating the aqueous protein solution from residual canola oil seed meal,
- (f) increasing the protein concentration of said aqueous protein solution while maintaining the ionic strength substantially constant by using a selective membrane technique to provide a concentrated protein solution,
- (g) diluting the concentrated canola protein isolate into chilled water having a temperature of below about 15°C to cause the formation of discrete protein particles in the aqueous phase in the form of micelles,
- (h) settling the protein micelles to form an amorphous, sticky, gelatinous, gluten-like protein micellar mass,
- (i) separating the protein micellar mass from supernatant and drying the separated protein micellar mass to provide a first canola protein isolate having a protein content of at least about 90 wt% ( $N \times 6.25$ ), and
- (j) following separation of the protein micellar mass therefrom, the supernatant is processed, on a batch, semi-continuous or continuous basis, to recover therefrom a second canola protein isolate having a protein content of at

Application No. 10/561,430  
Amtd. Dated: March-21-2011  
Reply to Office Action: Dec-21-2010

least about 90 wt% (N x 6.25), and wherein said several second protein isolate is obtained by:

- (a) concentrating the supernatant to a protein concentration of about 100 to about 400 g/L and drying the concentrated supernatant; or
- (b) concentrating the supernatant to a protein concentration of about 100 to about 400 g/L mixing the concentrated supernatant with the recovered protein micellar mass, and drying the mixture; or
- (c) concentrating the supernatant to a protein concentration of about 100 to about 400 g/L mixing a portion of said concentrated supernatant with at least a portion of the recovered protein micellar mass, and drying the resulting mixture.

28. (Previously presented) The method of claim 27 wherein, in option (c), the remainder of the concentrated supernatant is dried and any remainder of the recovered protein micellar mass is dried.

29. (Previously presented) The method of claim 27 wherein the supernatant is concentrated to a concentration of about 200 to about 300 g/L.

30. (New) The method of claim 1 wherein the protein concentration of the aqueous protein solution is increased in step (iii) to at least about 250 g/L.

31. (New) The method of claim 12 wherein said extracting of said oil seed meal is effected using an aqueous salt solution having an ionic strength of about 0.15 to about 0.6 and a pH of about 5.3 to 6.2 to produce an aqueous protein solution having a protein content of about 10 to about 30 g/L

32. (New) The method of claim 14 wherein said extraction step is effected by:

- (i) continuously mixing said oil seed meal with an aqueous salt solution having an ionic strength of about 0.15 to about 0.6 and a pH of about 5.3 to about 6.2 at a temperature of at least about 35°C, and

Application No. 10/561,430  
Amdt. Dated: March-21-2011  
Reply to Office Action: Dec-21-2010

9

(ii) continuously conveying said mixture through a pipe while extracting protein from the oil seed meal to form an aqueous protein solution having a protein content of about 10 to about 30 g/L.

33. (New) The method of claim 16 wherein said extracting of said oil seed meal is effected using an aqueous salt solution having an ionic strength of about 0.15 to about 0.6 and, following separation of the aqueous protein solution from the residual oil seed meal, the pH of the aqueous protein solution is adjusted to a pH of about 5.3 to about 6.2.

34. (New) The method of claim 17 wherein said pigment removal step is effected by:

(a) diafiltration of the aqueous protein solution, or

(b) mixing a pigment adsorbing agent with the aqueous protein solution and subsequently removing the pigment adsorbing agent from the aqueous protein solution.

35. (New) The method of claim 19 wherein said concentrated protein solution is warmed to a temperature of about 25°C to about 40°C to decrease the viscosity of the concentrated protein solution.

36. (New) The method of claim 20 wherein said diafiltration is effected using about 2 to about 20 volumes of diafiltration solution until no further quantities of phenolics and visible colour are present in the permeate.

37. (New) The method of claim 36 wherein about 5 to about 10 volumes of diafiltration solution are employed.

38. (New) The method of claim 21 wherein the anti-oxidant is sodium sulfite or ascorbic acid present in an amount of about 0.01 to about 1 wt%.

Application No. 10/561,430  
Amdt. Dated: March-21-2011  
Reply to Office Action: Dec-21-2010

10

39. (New) The method of claim 22 wherein said pigment removal step is effected using powdered activated carbon in amount of about 0.025 to about 5% w/v or using polyvinylpyrrolidone in an amount of about 0.5 to about 5 wt%.

40. (New) The method of claim 39 wherein said activated carbon is used in an amount of about 0.05 to about 2 wt% or said polyvinylpyrrolidone is used in an amount of about 2 to about 3 wt%.

41. (New) The method of claim 23 wherein said pasteurization step is effected by heating the concentrated and optionally diafiltered protein solution at a temperature of about 60° to about 65°C for about 10 to about 15 minutes and wherein, following pasteurization step, the pasteurized solution is cooled to a temperature of about 25° to about 40°C.

42. (New) The method of claim 24 wherein said concentrated protein solution is diluted by about 10 fold or less of chilled water having a temperature of less than about 10°C.

43. (New) The method of claim 25 wherein the concentrated protein solution is diluted by about 10 fold or less of chilled water having a temperature of less than 10°C.